Bamifylline, a xanthine derivative with bronchodilator properties, is used in the treatment of asthma and reversible airway obstructions. The physico-chemical and pharmacokinetic properties of bamifylline differ from those of theophylline (I).

We describe an isocratic reversed-phase HPLC method with detection at 276 nm for determination of bamifylline and three of its metabolites in human plasma, with fenetyl- line as internal standard.

The column is an Ultrasphere (Altex, Berkeley, CA), 25 cm × 4.6 mm (i.d.), 5-μm particle diameter. The mobile phase is prepared by mixing 50 mL of acetonitrile with 950 mL of 0.1 mol/L KH2PO4 containing 10 g of tetrabutylammonium hydrogen sulfate (TBA) per liter and adjusted to pH 2.5. The lipophilic compounds are strongly retained on the octadecyl-bonded silica when the mobile phase is a mixture of phosphate buffer and acetonitrile without added TBA. The added TBA decreases the retention of all compounds, as expected from the ion-interaction model proposed by Bidlingmeyer et al. (2). A TBA concentration of 10 g/L results in near-baseline resolution of the different compounds. The number of theoretical plates (N), the capacity factor (k'), the asymmetry factor (AF) for the various compounds, and the resolution (R) between vicinal peaks are listed in the following tabulation. Chromatographic run time is 26 min.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>k'</th>
<th>AF*</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bamifylline</td>
<td>14 200</td>
<td>5.1</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>AC 85</td>
<td>13 200</td>
<td>5.6</td>
<td>0.8</td>
<td>2.1</td>
</tr>
<tr>
<td>AC 119</td>
<td>15 000</td>
<td>6.4</td>
<td>1.0</td>
<td>2.9</td>
</tr>
<tr>
<td>AC 155</td>
<td>15 100</td>
<td>7.3</td>
<td>0.9</td>
<td>2.7</td>
</tr>
<tr>
<td>Fenetyl line</td>
<td>14 100</td>
<td>7.7</td>
<td>1.0</td>
<td>1.5</td>
</tr>
</tbody>
</table>

*Measured at 10% of peak height.

Bamifylline and its metabolites are extracted from 1 mL of plasma by a procedure adapted from Nicot et al. (3). The mean (and SD) absolute recovery (n = 16) of fenetyl line averaged 90.4% (3.7%). Analytical recovery of bamifylline, expressed as percentage of the recovery of the internal standard, was 99.2% (4.9%). For the metabolites AC 85, AC 119, and AC 155, the relative recovery was, respectively, 79.8% (6.1%), 95.3% (6.5%), and 83.9% (8.4%). The assay curve is linear over the concentration range studied (0.1–5.0 mg/L). The between-run CV (n = 7) was <6% except for AC 155 (12%).

The assay is used for monitoring bamifylline in neonates.

References


Intermediary metabolites in blood are usually measured by deproteinizing the blood with perchloric acid, neutralizing the resulting extract with a potassium salt (to precipitate the insoluble potassium perchlorate), and enzymatic analysis. Some metabolites (particularly pyruvate and acetate) are very labile in blood, so it is common practice to add blood, straight from the syringe, to a weighed tube containing perchloric acid. The weight of the added blood is found by difference. The supernate after centrifugation is removed and weighed, then reweighed after manual neutralization with KOH or K2CO3 solution. Several authors have now reported micro-methods for the enzymatic assays themselves (1, 2), but the sample preparation is laborious and not easily miniaturized. We describe here a simple micro-method for performing these preparative stages.

We prepare microcentrifuge tubes (1.5 mL) by adding 200 μL of 70 g/L HClO4 (58.8 g HClO4, relative density 1.54, diluted to 500 mL) with a repeating pipette, and store them at 4°C until required. Blood is sampled into a syringe and delivered into a heparinized microcentrifuge tube. With a positive-displacement pipette, 100 μL is transferred into the prepared perchloric tube, which is capped and vortex-mixed. The remaining heparinized blood is centrifuged to prepare plasma for other assays.

We store the tubes at 4°C until assayed. Then we centrifuge them (3600 × g, 20 min, 4°C), transfer 200 μL of supernate to a clean 1.5-mL microcentrifuge tube, add 60 μL of an equilibrium mixture of KHCO3 (1 mol/L) and K2CO3 (1 mol/L), mix, and leave the tube on ice (un capped to allow CO2 to escape) for 10–30 min to ensure complete precipitation of KClO4. The capped tubes are then centrifuged (1500 × g, 10 min, 4°C) and the clear, neutral supernatant fluid is used for enzymatic assays. We do not remove it from the pellet.

Final results are multiplied by the dilution factor, 3.9 = (200 + 100/100 × (260/200)).

We tested the method on blood samples with added glucose, lactate, 3-hydroxybutyrate, and glycerol. Four replicate extractions were done, as above, before this addition (to measure endogenous concentrations), and 10 replicate extractions after addition. Respective mean (and SD) analytical recoveries were 102 (3%), 102 (3%), 112 (4%), and 97 (5%). (We cannot explain the high recovery of 3-hydroxybutyrate.)

We run micro-enzymatic assays for these analytes in an IL FLS Multistat III Micro Centrifugal Analyzer and will willingly provide details of these on request.

References

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